

Example 14: Cross-Linking of Soluble P-Selectin-Fc Fusion Protein Induces Apoptosis of Activated T Cells

5 Mouse selectins (P-Selectin, E-Selectin, and L-Selectin) were fused to the Fc region of human IgG1 as detailed above to form soluble dimeric fusion proteins. To evaluate whether the soluble selectins can induce apoptosis of activated T cells, an experiment was performed as detailed in the in Example 13, with the exception that the plate-bound anti-human Fc Ig was omitted. Negligible or low levels of apoptosis of activated T cells occurred in the presence of the soluble form of P-selectin fusion protein (a dimer) alone (Fig. 12).
10 However, upon the addition of a cross-linker (anti-human Fc) the apoptotic activity increased substantially, to approximately the apoptotic level induced in the presence of the plate-bound antibody. Neither anti-human Fc, human Ig (HIg), nor BSA induced apoptosis.

Similar results were obtained for the E-selectin-Fc fusion protein as were obtained for the P-selectin-Fc fusion protein. In addition, consistent with the results obtained for the
15 plate-bound (multimeric form) of L-selectin, the soluble form of L-selectin fusion protein did not induce apoptosis of activated T cells.

Other Embodiments

----- It is to be understood that, while the invention has been described in conjunction with
20 the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention. Other aspects, advantages, and modifications of the invention are within the scope of the claims set forth below.

What is claimed is:

1. A method of preventing or reducing a T cell-mediated immune response in an individual, the method comprising:

selecting an individual diagnosed as having or as being at risk of acquiring a condition characterized by an excessive or unwanted T cell-mediated immune response; and

5 administering to the individual a multimeric compound that binds to at least two P-Selectin Glycoprotein Ligand 1 (PSGL-1) proteins on the surface of a T cell, wherein the multimeric compound comprises two polypeptide chains, each of the polypeptide chains comprising (i) a binding domain that binds to PSGL-1, and (ii) a heterologous amino acid sequence, wherein the polypeptide chains are linked via the heterologous amino acid
10 sequence to form the multimeric compound,

wherein the binding of the multimeric compound to the at least two PSGL-1 proteins on the surface of the T cell induces a signal transduction pathway that results in the death of the T cell, thereby preventing or reducing a T cell-mediated immune response in the individual.

15 2. The method of claim 1, wherein the multimeric compound is a homo-multimeric compound.

~~3. The method of claim 1, wherein the multimeric compound is a hetero-multimeric~~
20 ~~compound.~~

4. The method of claim 1, wherein the heterologous amino acid sequence comprises a cell surface receptor binding region.

25 5. The method of claim 1, wherein the binding domain comprises a P-Selectin extracellular domain or a PSGL-1-binding fragment thereof.

6. The method of claim 1, wherein the binding domain comprises an E-Selectin extracellular domain or a PSGL-1-binding fragment thereof.

7. The method of claim 1, wherein the binding domain comprises an L-Selectin extracellular domain or a PSGL-1-binding fragment thereof.

8. The method of claim 1, wherein the binding domain comprises an antigen binding domain of an anti-PSGL-1 antibody or a fragment thereof.

9. The method of claim 1, wherein the binding domain comprises a PSGL-1 binding polypeptide selected from a phage display library.

10. The method of claim 1, wherein the polypeptide chains are covalently linked via the heterologous amino acid sequence to form the multimeric compound.

11. The method of claim 10, wherein the covalent linkage is a disulfide linkage.

12. The method of claim 1, wherein the heterologous amino acid sequence comprises an immunoglobulin heavy chain constant region.

13. The method of claim 1, further comprising administering to the individual an agent that binds to the multimeric compound via the heterologous amino acid sequence and induces cross-linking of a plurality of PSGL-1 antigens on the surface of the T cell.

14. The method of claim 1, comprising selecting an individual diagnosed as having an inflammatory disease.

15. The method of claim 1, comprising selecting an individual diagnosed as having an autoimmune disease.

16. The method of claim 1, comprising selecting an individual that has received or is expected to receive an allogeneic or xenogeneic transplant.

17. The method of claim 1, comprising selecting an individual diagnosed as having an allergic disease.

18. The method of claim 1, comprising selecting an individual diagnosed as having a T cell cancer.

19. The method of claim 1, wherein the T cell is an activated T cell.

20. The method of claim 1, wherein the method comprises detecting the number of T cells in a first biological sample taken from the individual before the administration of the multimeric compound and comparing the results with the number of T cells in a second biological sample taken from the individual after the administration of the multimeric compound.

21. The method of claim 1, wherein the method comprises detecting a biological activity of T cells in a first biological sample taken from the individual before the administration of the multimeric compound and comparing the results with the biological activity of T cells in a second biological sample taken from the individual after the administration of the multimeric compound.

22. The method of claim 1, wherein the administration results in the depletion of at least 10% of activated T cells in the individual.

23. A method of inducing the death of a T cell or a natural killer (NK) cell, the method comprising:

providing a T cell or NK cell expressing PSGL-1 on its cell surface; and

contacting the T cell or NK cell with a multimeric compound that binds to at least two PSGL-1 proteins on the surface of the T cell or NK cell, wherein the multimeric compound comprises two polypeptide chains, each of the polypeptide chains comprising (i) a binding domain that binds to PSGL-1, and (ii) a heterologous amino acid sequence, wherein the

polypeptide chains are linked via the heterologous amino acid sequence to form the multimeric compound,

wherein the binding of the multimeric compound to the at least two PSGL-1 proteins on the surface of the T cell or NK cell induces a signal transduction pathway that results in the death of the T cell or NK cell.

24. The method of claim 23, wherein the multimeric compound is a homomultimeric compound.

25. The method of claim 23, wherein the multimeric compound is a heteromultimeric compound.

26. The method of claim 23, wherein the heterologous amino acid sequence comprises a cell surface receptor binding region.

27. The method of claim 23, wherein the binding domain comprises a P-Selectin extracellular domain or a PSGL-1-binding fragment thereof.

28. The method of claim 23, wherein the binding domain comprises an E-Selectin extracellular domain or a PSGL-1-binding fragment thereof.

29. The method of claim 23, wherein the binding domain comprises an L-Selectin extracellular domain or a PSGL-1-binding fragment thereof.

30. The method of claim 23, wherein the binding domain comprises an antigen binding domain of an anti-PSGL-1 antibody or a fragment thereof.

31. The method of claim 23, wherein the binding domain comprises a PSGL-1 binding polypeptide selected from a phage display library.

32. The method of claim 23, wherein the polypeptide chains are covalently linked via the heterologous amino acid sequence to form the multimeric compound.

33. The method of claim 32, wherein the covalent linkage is a disulfide linkage.

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34. The method of claim 23, wherein the heterologous amino acid sequence comprises an immunoglobulin heavy chain constant region.

35. The method of claim 23, further comprising contacting the multimeric compound
10 an agent that binds to the multimeric compound via the heterologous amino acid sequence and induces cross-linking of a plurality of PSGL-1 antigens on the surface of the T cell.

36. The method of claim 23, comprising inducing the death of an activated T cell.

37. The method of claim 23, wherein the method comprises assessing the viability of
15 the T cell or NK cell after the contacting with the multimeric compound.

38. The method of claim 23, wherein the method comprises assessing a biological
activity of the T cell or NK cell after the contacting with the multimeric compound.

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39. A kit comprising:

a multimeric compound that binds to at least two PSGL-1 proteins on the surface of a T cell, wherein the multimeric compound comprises two polypeptide chains, each of the polypeptide chains comprising (i) a binding domain that binds to PSGL-1, and (ii) a
25 heterologous amino acid sequence, wherein the polypeptide chains are linked via the heterologous amino acid sequence to form the multimeric compound, wherein the binding of the multimeric compound to the at least two PSGL-1 proteins on the surface of the T cell induces a signal transduction pathway that results in the death of the T cell; and

instructions for use of the multimeric compound to treat inflammation, autoimmunity,
30 transplant rejection, an allergic condition, or a T cell cancer.